I hereby certify that this correspondence is being sent by facsimile transmission in accordance with § 1.6(d) addressed to Art Unit 1644, Central Facsimile No. (703) 872-9306, the Commissioner for Patents, P.O Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Date: August 26, 2004 By: Aida Martinez

PATENT
Docket No. GC527C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
D. A. Estell *et al.*	Group Art Unit: 1644
Serial No.: 09/677,822	Examiner: Saunders, D.
Filed: October 2, 2000	
For: Proteins Producing an Altered Immunogenic Response and Methods of Making and Using the Same	

DECLARATION OF DAVID ESTELL AND FIONA HARDING UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. We, David Estell and Fiona Harding, are co-inventors of the subject matter embodied in the above-identified patent application.

2. We have read and understand the above-identified patent application, which was filed on October 2, 2000; the priority patent applications Application Serial Number 09/500,135, filed February 8, 2000, and Application Serial Number 09/060,872, filed April 15, 1998; all of the Claims as amended and filed in the "Amendment and Response to Office Action" filed herewith; the Office Action from the U.S. Patent & Trademark Office, mailed June 3, 2004; and the references by Landry *et al.* (WO 99/06061; published February 11, 1999), and Grieve *et al.* (U.S. Pat. No. 6, 060,281; issued May 9, 2000).

3. The work that is the subject of the pending Claims and that is described in paragraphs 4 to 11 below, was performed in this country either by us or under our supervision.

4. Prior to February, 1999, we successfully carried out assay experiments as described in the present patent application, in which the amino acid sequence of a T-cell

epitope peptide was modified to increase the magnitude of the induced proliferative response. A peripheral blood (PBMC) sample from a Genencor employee who was verified by Genencor's Environmental Health and Safety department as sensitized to *B. lentus* subtilisin was drawn by the Stanford University Blood Center. Monocytes from the PBMC sample were cultured with GM-CSF and IL-4 for 5 days in order to cause the differentiation of dendritic cells (DC). IL-1 and TNF-alpha were subsequently added, and the DC cultures were incubated for another 2 days. The final DC cultures were harvested on day 7. On day 7, CD4+ T cells from the donor PBMC sample were isolated from frozen aliquots.

5. Peptides encompassing the amino acids 160-174 from *B. lentus* subtilisin, and a series of alanine scan peptide variants, were purchased from Mimotopes.

6. CD4+ T cells and DC from the sensitized donor were co-cultured with either the unmodified parent peptide, or the alanine substituted variant peptides. Cultures were incubated for 5 days. On day 5, 0.5 uCi of tritiated thymidine was added to each well of the cell culture. On day 6, the cell cultures were harvested to glass fiber mats, and incorporated tritiated thymidine was measured.

7. This donor had been previously shown to respond to the amino acid 160-174 region of *B. lentus* subtilisin by mounting a CD4+ T cell response. In this experiment, the donor again responded to the unmodified amino acid 160-174 peptide. The stimulation index of the proliferative response (experimental cpm divided by control well cpm) was about 7.

8. Responses to the alanine scan peptides were tabulated. Many of the alanine substituted peptides have no effect on the proliferative response (*i.e.*, the magnitude of the stimulation index to the variant peptide was approximately the same as the response to the unmodified parent peptide). Alanine changed peptides at some of the positions (R, Y, N) had a deleterious effect on the induction of a proliferation response. However, alanine substitutions at both positions #14 and #5 in the peptide resulted in proliferative responses that were more robust than the response to the unmodified parent peptide.

9. Please see Figure 11 in the application as filed, which shows the increased proliferative response to variant peptide carrying changes #5 and #12 as compared to the parent, unmodified peptide sequence.

10. In conclusion, this experiment shows that the modification of a T-cell epitope peptide sequence can result in an increased proliferative response to the polypeptide.

11. These experiments are described on pages 89-99 of Fiona Harding's laboratory notebook number 1471, attached hereto.

The undersigned declares further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 19 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom

Dated: 26 Aug 2004 Signed: _____
DAVID ESTELL

Dated: 26 August 2004 Signed: _____
FIONA HARDING